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THE BASIC OBJECTIVES OF THIS RESEARCH were to determine if transcription (and resulting translation) is altered in human cells exposed to extremely low frequency (elf) electromagnetic fields (EMFs), and if transcription is altered, are there characteristics of elf EMFs or cells that can related to effect? **TRANSCRIPTION.** RNA transcripts are increased following short exposures (less than 40 minutes) of human HL60 cells to elf EMFs. EFFECT OF AMPLITUDE/TIME. Defined transcripts were quantitatively measured following exposure of HL60 cells to sine signals at 60 Hz. Globin mRNA, not usually expressed in HL-60 cells was not expressed in either control or exposed cells. Other transcripts showed a significant increase which was dependent on amplitude and/or time. EFFECT OF FREQUENCY. HL60 cells were exposed for 20 minutes to continuous sinusoidal electromagnetic signals at 6 frequencies. The most pronounced increase in each transcript occurred with exposure to 45 Hz. COMPARISON OF SINUSOIDAL SIGNALS WITH ASYMMETRIC WAVEFORMS. The effect of three asymmetric signals was compared to sinusoidal waveforms at both 60 and 72 Hz. Short exposures of HL60 cells to any of the five signals caused an increase in the transcripts measured. The augmentation of transcript, however, is signal dependent. Cells exposed to sinusoidal signals show the greatest transcript increase. **TRANSLATION.** THE EFFECT OF ELF EMFS ON PROTEIN SYNTHESIS. 2-D gels were run using polypeptides from human HL60 cells exposed to 60 and 72 Hz signals at amplitudes of 0.5, 5, 50 and 500 uV for 45 minutes. Three features were noted: (1) the correlation coefficients show that the experimental and control profiles are different; (2) quantitative differences are observed in selected molecular weight classes, and (3) quantization of proteins supports data which measured quantity of RNA transcripts at the above amplitudes.

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EXPOSURE OF HUMAN CELLS TO ELECTROMAGNETIC FIELDS

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ABSTRACT

This study addressed the following basic question: How does extremely low-level non-ionizing radiation affect human cells, and if there are cellular responses that can be directly related to signal parameters such as frequency, amplitude and time of exposure? The focus of these studies was to identify transcriptional changes in human cultured cells, HL60, which result from exposure of these cells to defined extremely low frequency electromagnetic fields (elf EMFS). Our experiments show a pronounced measurable response observed as transcript increase, with associated changes in protein synthesis. The major findings relative to transcriptional changes are fourfold: (1) transcript changes in human cells correlate with previous findings of transcriptional and translational changes in Drosophila salivary gland cells; (2) the frequency of the signal in the range from 15 to 150 Hz results in a "window" at 45 Hz; (3) changing the amplitude (with resulting changes in E- and B-fields) in log increments from 0.5 to 500 uV at 60 Hz gives both amplitude and time-dependent windows, and (4) genes not usually expressed in HL-60 are unaffected by exposure to elf EMFs. Changes in the overall protein synthetic pattern have also been observed following exposure of HL60 cells to 60 Hz signals.

1.0 INTRODUCTION

1.1 GOALS OF THE PROJECT. The purpose of this project is to determine how extremely low frequency (elf) electromagnetic fields (EMF) affect transcription in human cells. Associated with this goal is the development of a consistent testing system for unequivocal identification of deviations in human cells caused by exposure to elf EMFs. The problem can be considered from two viewpoints. The first is to determine if specific characteristics of elf EMFs that can be related to observed effects, and the second, the nature of the cellular mechanisms that directly

interact with low frequency non-ionizing radiation; *i.e.*, what factors within the cell respond or initiate the response to elf EMFs. Our studies have concentrated on determining which events are affected within the cell on the hypothesis that defining a specific event will expedite an understanding of how the cell responds.

1.2 BACKGROUND. Extremely low frequency (elf) electromagnetic fields (EMFs) cause changes in vivo or in vitro in a variety of biological systems. Elf EMFs are variously described by different investigators as low enough to be non-thermal, signals less than 200 to 300 Hz, and having wavelengths larger than the dimensions of the structure with which interaction occurs. Clinically, elf emfs have been used successfully in the healing of recalcitrant bone non-unions (1). Environmentally, elf EMFs have been implicated in deleterious health effects as identified by epidemiological studies (2,3).

The apparent lack of consensus on any viable mechanistic explanation, has hampered acceptance of any elf EMF interaction with cells. There are at least two major problems. On a practical level, there is a vested interest in assuring the general public that the electrical environment is not hazardous. The second problem is more compelling. At the present time, there are no known cellular mechanisms that can explain the myriad effects reported as resulting from exposure of cells to elf EMFs; observed cellular effects following exposure to elf EMFs are viewed with caution by many biologists as impossible in light of what is known about the cell and cell membrane potential. Models based on ionizing radiation are inadequate, since there is no significant transfer of energy from EMF to cells, as observed in ionizing radiation. The controversy is augmented in interdisciplinary interactions by the failure to find a meaningful physical route for such interactions to occur. Recently, the Office of Technology Assessment completed a remarkably unbiased review of all the research published with respect to elf field effects (4). Some examples of these effects at the cellular level include modulation of ion and protein flow across cell membranes, interference with DNA synthesis, alterations in RNA transcription, interaction with the response of normal cells to hormones, neurotransmitters and growth factors, interaction with the kinetics of cancer cells, as well as (possibly associated) changes in calcium flux. In light of reported effects and disagreement as to how such effects arise, it is critical to determine how the cell responds to electromagnetic fields. This is particularly important when intensities as low as 1-100 milligauss (mG) are involved.

Explanations of mechanisms are complicated by the fact that so many forms of electric, magnetic or electric and magnetic fields are in use experimentally. Some investigators use RMF modulated to sinusoidal levels in the 5 to 100 Hz range. Other investigators use complicated waveforms inspired in part by signals used in commercial bone healing apparatuses. The asymmetric signals contain a broad band of frequencies. The repetition rates are within the elf range, but the frequency content of the asymmetric signals can range to 10 MHz (5). The time of exposure and the cell type investigated also vary.

In spite of the problems involved, working models have been devised to explain interactions between cells and electromagnetic fields. Some support the concept of interaction at the transcriptional level. Any model, however, must take into account the energy level of the nonionizing radiation that results in measurable biological effects. Elf EMFs are generally too low to act through known physical mechanisms of heating, dielectric breakdown, particle displacement or electrophoresis (6). Some investigators consider it unlikely that the mechanism of action is derived simply from transmembrane potentials, since signal potentials are much lower than cell membrane potentials. Speculation has concentrated, for the most part, on postulating some form of coupling, resonance, or other interaction with endogenous processes.

We have made an extensive effort to obtain an experimental model that can ultimately be used as a means of determining cellular interaction with defined elf EMFs. Our original assumption was that any measurable change in the cell should be reflected in transcriptional patterns. Associated with the experimental model is a time factor. If transcription is affected, the response should be observed for many transcripts in a matter of minutes, rather than hours. Finally, the response should have a relationship to signal parameters. Each of these has been shown to be the case in our research.

2.0 EXPERIMENTAL CONDITIONS

2.1 EXPOSURE OF CELLS TO ELFS EMFS. Characteristics of the cells. Cultured cells from the lymphocytic cell line, HL60, were used in all experiments (7). This line, established from peripheral circulating cells of a patient with acute promyelocytic leukemia, consists of about 90% myeloblasts and promyelocytes and 10% differentiated myeloid or monocytic cells. HL60 cells were selected for the experiments for several reasons: (a) they are capable of undergoing morphological and functional

differentiation in response to a number of chemicals (8-10); (b) the c-myc gene is amplified (11-13) and c-myc hybridizing sequences in the poly (A)-containing mRNA have been demonstrated (14), and (c) the cells are rapidly dividing which simplifies cell culture and will ultimately allow determinations of transcripts which are related to growth characteristics.

Characteristics of electromagnetic signals. Three types of elf electromagnetic signals generating asymmetric pulsed fields are used: SP (Bio-steogen system 204; Electro-Biology, Inc.) with a single pulse rate of 72 Hz, PT (Biosteogen system 204; Electro-Biology, Inc) with a burst repetition rate (pulse train) of 15 Hz and E33 (pulse-train; battery powered, manufactured by Electro-Biology, Inc. as Biosteogen System 100367) with a repetition rate of 1.5 Hz. Two symmetric sinusoidal signals, generating 60 (CW60) and 72 (CW72) Hz, are also used. The characteristics of these signals is given in Figures 1 and 2.

Monitoring Signal Generating Equipment: Magnetic flux density, field uniformity, harmonic content, stray magnetic fields, DC magnetic fields, induced current density, absence of vibrations and warming of the coils are monitored. The search coil used for calibrations is a 3 cm diameter 21x probe designed by Electro-Biology, Inc. Signal-generating equipment is monitored daily by the staff, weekly by Mr. Rick Cangiolosi (Engineering, EBI), and on a routine basis by an in-house consultant. Twice in the last year, outside consultants (Drs. M. Misakian and F. Dietrich) checked all equipment, and monitored for possible outside sources of EMFs. Dr. C. Blackman measured the geomagnetic field. The local geomagnetic field at the sample location is 8.2 uT, with an inclination of 12.7°N. The signals are monitored before and after the exposure of the cells with a Tektronix 2465 (300 MHz) oscilloscope using a calibrated search coil (21x).

Exposure conditions. Sinusoidal signals are generated by a Wavetek signal generator and Realistik (RadioShack) amplifier. 10 x 10 cm Helmholtz-aiding coils are used. Signal parameters are tested before and after experiments. The sine wave generator is used with a variable frequency control. Field parameters are measured under the same geometrical and experimental conditions at the location and in the absence of the experimental sample. Temperatures in the surrounding medium are monitored in parallel experiments using a thermometer attached to an external chart drive, to avoid perturbing the field. The spatial distribution of the induced field is discussed in McLeod et al. (15). Either set of coils can be alternatively used as experimental or control fixtures. Periodically, the roles are reversed or used simultaneously as controls. The coils are

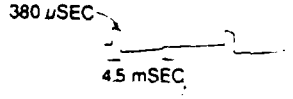
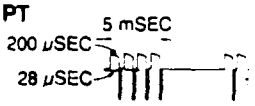
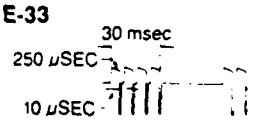
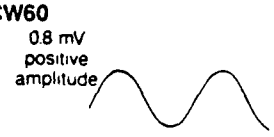
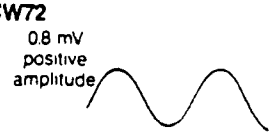
Signal	Waveform	Rate Hz	Positive Induced Amplitude mV	Positive Duration µsec	Burst Width msec	Negative Space µsec	Negative Spike µsec	Peak Magnetic Field mT
SP		72	15	380			4500	3.5
PT		15	14.5	200	5	28	24	1.9
E-33		1.5	2.5	250	30	10	4	0.38
CW60		60	0.8					1.5
CW72		72	0.8					1.1

Figure 1. Major waveform characteristics of the electromagnetic signals used for signal comparisons. Frequency content of the three asymmetric pulses differs significantly as derived by discrete Fourier transforms (5).

AMPLITUDE	0.5uV	5uV	50uV	500uV
dB/dT [Tesla/sec]	3×10^{-4}	3×10^{-3}	3×10^{-2}	3×10^{-1}
PEAK B FIELD [Teslas]	8×10^{-7}	8×10^{-6}	8×10^{-5}	8×10^{-4}
E FIELD[a] [volts/meter]	3×10^{-7}	3×10^{-6}	3×10^{-5}	3×10^{-4}
E FIELD[b] [volts/meter]		15×10^{-6}		

Figure 2. Parameters of the 60 Hz signal used in amplitude studies. [a]In H_20 at 2mm radius and circular path as estimate of magnitude; [b]Independent measurement in laboratory of T. Litowitz.

constructed of wire bundles approximately one cm in diameter wound around a square form with a 10 cm distance between the sides. There is a 7.5 cm radius from the center of the form. The coils are positioned with their central axis horizontal; samples are placed on a plexiglass stand in the horizontal plane in an area shown to have a uniform magnetic field and maximum field strength.

All experiments use the same exposure conditions. Cells (5×10^5/ml) in 10 mls of RPMI (10% fetal calf serum) are exposed in a 37.5°C incubator in 25 cm^2 flasks. Heat shock cells (42°C) serve as internal controls for heating effects (characteristics of heat shock have not been observed). Cells for each experimental series are grown in the same flask. They are allocated to separate flasks at the same cell density 60 minutes before experiments are initiated. Flasks are kept in the incubator in a separate (left) compartment in a area known to have minimum stray fields (which remain unchanged during exposures) until they are used. Exposures occur in the right separate compartment of the incubator. The water-jacketed incubator is unplugged during experimental periods, but the incubator temperature remains constant. All exposures are in a large sealed mu metal container to eliminate the possibility of stray fields. All samples are coded. Two internal controls are used for each experiment. For control 1, the flask is placed in the incubator in a small mu metal box for appropriate time periods prior to initiation of the experiments. Control 2 is sham exposed cells (done the middle of the experiments) where the cells are placed in the mu metal container with the coil present, but disconnected from the function generator. There are no differences between the values in controls 1 and 2.

2.1 EXPERIMENTAL ANALYSIS OF TRANSCRIPTS. Hybridization analyses use dot blots for quantization of transcripts. Since quantification in any biological system is always open to criticism, we are using the best possible means of analysis via a quantitative dot blot system, and duplicating our measurements using two independent systems-fine tuned densitometry, as well as direct counting of the radioactive spot. We use Northern blotting procedures to test size of transcript. The distinct advantage of the dot blot system, however, is in the dilution procedures. Essentially, the RNA spotted onto the nitrocellulose paper is diluted to a point where no signal can be visualized in the control (or other) preparations. If a signal persist in "experimental" RNA preparations at the dilution point where none is apparent in the controls, it is an excellent (and normally used) indication that we are achieving an increase in RNA

transcripts. The second indication that our procedures are adequate is in the linearity of measured points relative to the dilution, *i.e.*, each series of dilutions for individual experiments is plotted relative to other points in the series. If the linear response over a series of dilutions is greater, we have confirmation that an increase in transcription has occurred. At least three replicate experiments are done measuring each experimental point. This is to ensure that the results we obtain are not due to factors related to inter-experimental variations.

The probes used for hybridizations are histone (H2B), β-actin, v-src and v-myc DNA (Oncor), which were cloned from chicken DNA, but have extensive homology to corresponding human genes; human α-globin DNA (obtained from A. Bank, Columbia Health Sciences), and human β-tubulin DNA (from the Repository of Human DNA Probes and Libraries). DNA probes are labelled *in vitro* with ^{32}P dCTP or ^{32}P dATP (NEN) (16); specific activities are between 5 and 10 x 10^7 cpm/ug. All RNA samples are tested by electrophoresis on agarose gels to assure that no breakdown of the sample occurred. Radioactive measurements (cpms) use cut pieces of the nitrocellulose filters identified by autoradiography. Background counts use a random area of the filters where no radioactivity is present. Linearity relative to RNA concentration on the dot blots is determined by plotting RNA concentration (2,1,0.5, 0.25 and 0.125 ug/ml) vs. cpm for all experiments. The data is compared as percent total counts for either 1 or 2 ug points within a given experiment set. This is the most useful means of inter-experimental comparisons where DNA probes are of different specific activities. The general pattern for individual experiments, plotting cpms vs. parameter change, however, is the same as that obtained using % total counts.

2.2 EXPERIMENTAL ANALYSIS OF PROTEINS. Polypeptide patterns are obtained using 2-dimensional electrophoresis. Analysis of the 2-D gels is via a computerized matching program to standardize the sample numbers. Once matching is done, comparative analysis is simplified by concentrating on those experimental points which exceed 500 cpm, since as many as 12,000 total spots can be resolved in a given experimental series. The basic counts for control samples are aligned for comparison to determine the standard deviation between points expected to be identical. Experimental points are considered significantly different when the values exceed (by at least 10%) any value obtained in control experiments.

3.0 EXPERIMENTAL RESULTS

3.1 WHAT COMPONENTS OF EMF SIGNALS ARE CELLS RESPONDING TO; HOW COMPLICATED DOES THE SIGNAL HAVE TO BE?. COMPARISON OF SINUSOIDAL SIGNALS WITH ASYMMETRIC WAVEFORMS.

Results. The questions addressed were whether quantitative changes in defined transcripts can be measured in HL60 cells exposed to any of a series of distinctly different elf electromagnetic fields, and whether characteristics of the signal waveforms can be correlated with changes in the quantities of the transcripts. Three asymmetric signals in clinical use were compared to sinusoidal waveforms at both 60 and 72 Hz (17). The signals used in this study are representative of those used in medical settings and/or implicated in environmental effects. Three of the signals are in use medically or experimentally for treatment of non-union bone fractures, congenital pseudarthrosis, osteoporosis and osteonecrosis. Of the two sinusoidal signals, one approximates 60 Hz line current. The other, 72 Hz, is the same frequency as one of the asymmetric signals and could be used to test whether frequency, as one signal component, affected cellular response. Short time exposures of HL60 cells to any of the five signals causes an increase in levels of the transcripts measured. The augmentation of transcript levels, however, was signal dependent. Cells exposed to the two sinusoidal signals showed the greatest transcript increase. Actin, histone H2B and c-*myc* DNA were used as probes.

As seen in Figure 3, each signal induces a specific quantitative response in HL60 cells in modulating the basal levels of RNA transcripts with homology to β-actin, histone H2B and v-*myc* DNA. Five replicate experiments were done for all signals, using the same internal control for each experiment.

Conclusions. Analysis of experiments exposing HL60 cells to five different signals generating elf electromagnetic fields supported previous results that exposure of cells to EMFs can affect basal levels of transcripts. The endogenous quantities of RNA in HL60 cells with homology to β-actin, histone H2B and v-*myc* DNA were increased following 20 minutes exposure to the EMF signals. The relative increase was dependent on the signal characteristics. Exposure to symmetrical signals usually resulted in the highest transcript augmentation. Thus, complexity in the signal is not relevant to the affect on transcription; a increase in transcripts with homology to the DNA probes was observed in each experiments following exposure of cells to electromagnetic signals.

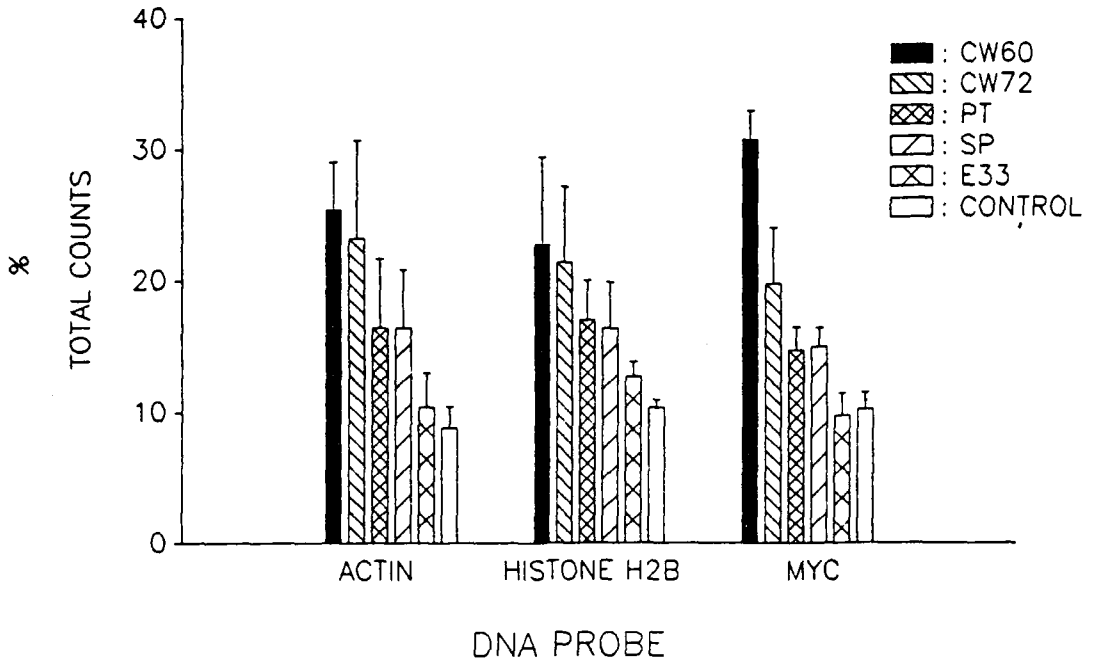


Figure 3. Analysis of dot blot hybridizations for relative transcript levels following exposure of HL-60 cells to five different electromagnetic fields. The histograms are of experiments where the cpm of bound [^{32}P] DNA (as indicated) in dot blot hybridizations exceeded 100. The mean of three experiments is given for actin and histone DNA, and 4 for c-*myc* DNA. Each experiment used points of 2, 1, 0.5, 0.25 and 0.125 *ug* total cellular RNA. The points for 2 *ug* are shown here. Bars are standard deviations. (from 17)

The exposure of HL60 cells to each of the signals resulted in an increase in RNA transcripts with homology to β-actin, as compared with unexposed control cells. There were differences, however, in the level of transcripts depending on the signal. The E33 signal (1.5 Hz) was the least effective. In some experiments, the effect of the E33 signal on the quantity of homologous transcripts was not consistently different from levels measured in unexposed control samples. Exposure of the cells to other signals with complex waveforms (PT and SP) resulted in significantly higher transcript levels relative to those observed in unexposed cells. There was some inter-experimental variability, but in general, cells exposed to the sinusoidal signals had the highest hybridization signals. C-*myc* transcripts were increased over control values following exposure of cells to each of the five electromagnetic signals. The pattern was similar to that observed for transcripts with homology to β-actin. The most effective signal was the CW60, with increasing quantities of c-*myc* transcripts as indicated by the following order: control < E33 < SP < PT < CW72.

Histone H2B transcripts were also quantitatively increased in cells exposed to each of the signals. There was no clear pattern of signal effectiveness considering all five experimental determinations, but three of the experiments gave results similar to that observed for transcripts with homology to v-*myc* DNA, *i.e.*, cells exposed to the sinusoidal signals had the highest level of transcript augmentation. The lack of correspondence among the five experiments may reflect the specificity of most histone synthesis to DNA replication, resulting in a variable level of overall synthesis in a asynchronous cell population.

A simple experimental design was used for this study. Five elf electromagnetic signals with defined wave forms were tested for their effect on transcript levels in HI cells. The result was an increase in transcript levels. On the basis of the present experiments, we were unable to determine the specific component(s) within the signals used which is responsible for the alteration in transcript quantity, other than the presence of an electromagnetic field. No direct correlation can be made between transcript increase and other parameters, such as positive induced amplitude (mV) or frequency.

The response of cells is different depending on whether asymmetric or symmetric fields are employed. A comparison of parameters between the asymmetric and symmetric signals is complicated since asymmetric signals contain a broad band of frequencies. The repetition rates are within the elf range (<100 Hz), but the frequency content of the asymmetric signals by discreet Fourier Transform ranges to 10 MHz (5). In the present experiments, transcript levels were higher in cells exposed to the CW72 signal as compared to the SP signal with the same repetition rate, but high frequency components within the SP signal could modulate the cellular response.

3.2 THE EFFECT OF FREQUENCY ON TRANSCRIPTION. Results. Human HL60 cells were exposed for 20 minutes to continuous sinusoidal electromagnetic signals at 5 different frequencies (15, 45, 60, 90 and 150 Hz) in four independent experiments (18). The amplitude was maintained at 500 uV. Two experiments also included exposure to a 72 Hz sinusoidal signal at 0.5 mV. Transcripts homologous to c-*myc* and histone H2B were measured by dot blot hybridization. The most pronounced increase in each transcript occurred with exposure to 45 Hz, resulting in levels more than four times those in unexposed control cells (Figure 4). The relative increase in c-*myc* and histone H2B transcripts gave identical patterns. This suggests that the cellular mechanism involved is a general one, and that

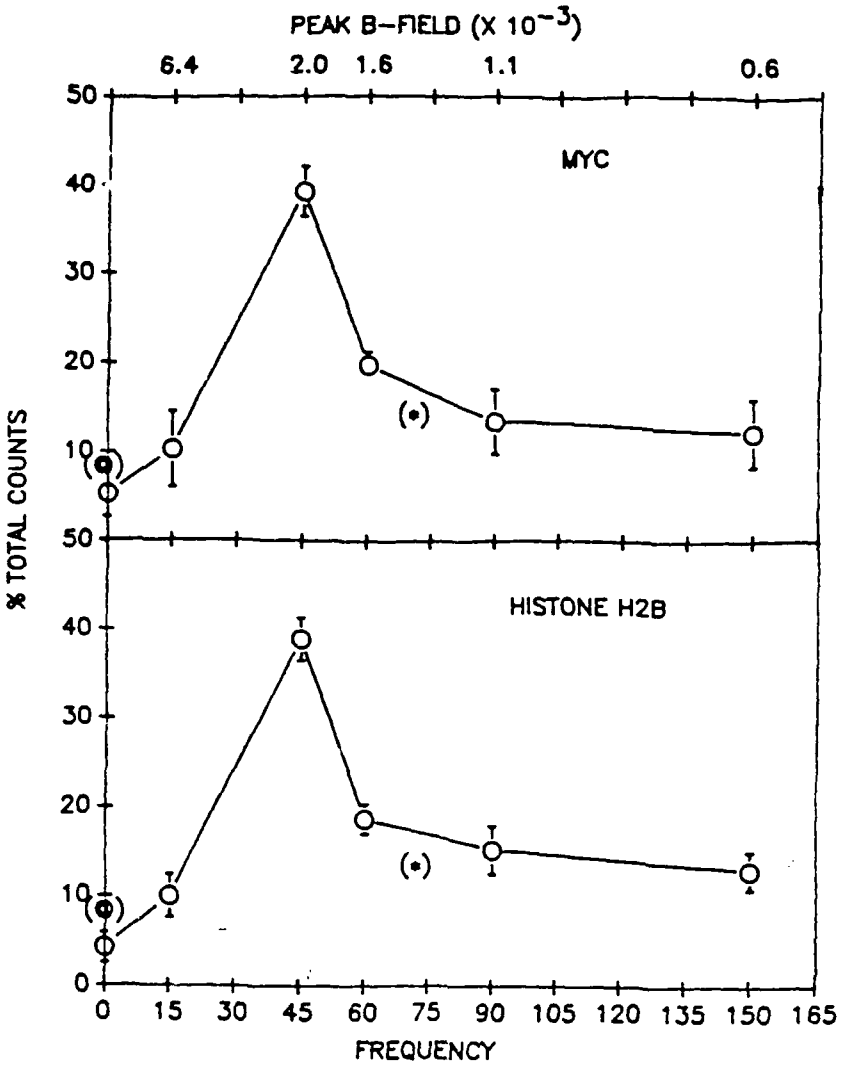


Figure 4. Analysis of the effect of frequency on quantity of specific transcripts in HL60 cells. Bars are standard deviations for the mean of four experiments. (@): represents the mean of two experiments where cells were heat-shocked at 42°C for 20 minutes; (*): the results of two experiments which used a frequency of 72 Hz. (from 18)

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a subset of genes respond similarly. Two experiments also included exposure to a 72 Hz sinusoidal signal at 500 uV, and RNA from cells that were heat shocked as an internal control.

Conclusions. The critical parameter in producing a window in the present experiments was frequency. It is of interest to identify window effects (19). Both inhibitory frequency windows (20-22) and windows where the effect is stimulated (23) have been reported. Frequency and

intensity windows have been observed previously (24); the initiating factors within the cell respond to conditions which are not directly proportional to dose. Blackman (24,25) demonstrated that sinusoidal fields can enhance the flux of calcium ions in a frequency-dependent manner.

3.3 THE EFFECT OF AMPLITUDE AND TIME OF EXPOSURE ON TRANSCRIPTIONAL CHANGES. Results. The effect of varying signal amplitude and exposure time on transcript levels in human HL60 cells has also been analyzed (26). An increase in the basal levels of normally expressed transcripts was observed with dependence on amplitude and time of exposure. In these experiments, HL60 cells were exposed to continuous sinusoidal fields with increasing amplitudes in log increments for periods of 10, 20 and 40 minutes to test the affect of time of exposure and changes in E- and B-field components on transcript levels. Two frequencies, 60 and 72 Hz, were tested at each of four amplitudes. Quantitative dot blot hybridizations were used to measure endogenous quantities of β-actin, histone H2B, α-tubulin, v-*src*, α-globin and c-*myc* transcripts (Figures 5 and 6). The level of α-globin transcripts was not significant in either exposed or unexposed cells. An increase in other transcripts over control levels was observed with dependence on signal parameter and time of exposure. The results were identical at the two frequencies used.

Rough estimates of copy number were made by comparison to measurements of *myc* transcripts in unexposed cells [estimated as 30 to 100 copies per cell (27)]. Our hybridization conditions detected about 30 c-*myc* transcripts per cell. Assuming a similar level of detection for the (smaller) globin transcript, there is less than one copy per cell in non-exposed cells, and 1 and 3 copies in exposed cells. The higher hybridization in RNA from exposed cells is assumed to be cross-hybridization with as yet an unidentified RNA. No hybridization on Northern blots was detected in the region of globin mRNA in unexposed or exposed cells.

Specific parameters of the signals are more effective. Increase in transcript levels was not proportional to either increasing time of exposure or amplitude. An amplitude and time frame window were observed, with a similar or the same pattern for each increased transcript, suggesting a broad regulatory response of the cell to elf EMFs (Figures 5 and 6). The initiating factors in the cell must respond prior to 10 minutes [preliminary experiments, using 50 uV at 60 Hz, indicate that the response may occur as early as 3 to 4 minutes]. The level of each transcript peaks when cells

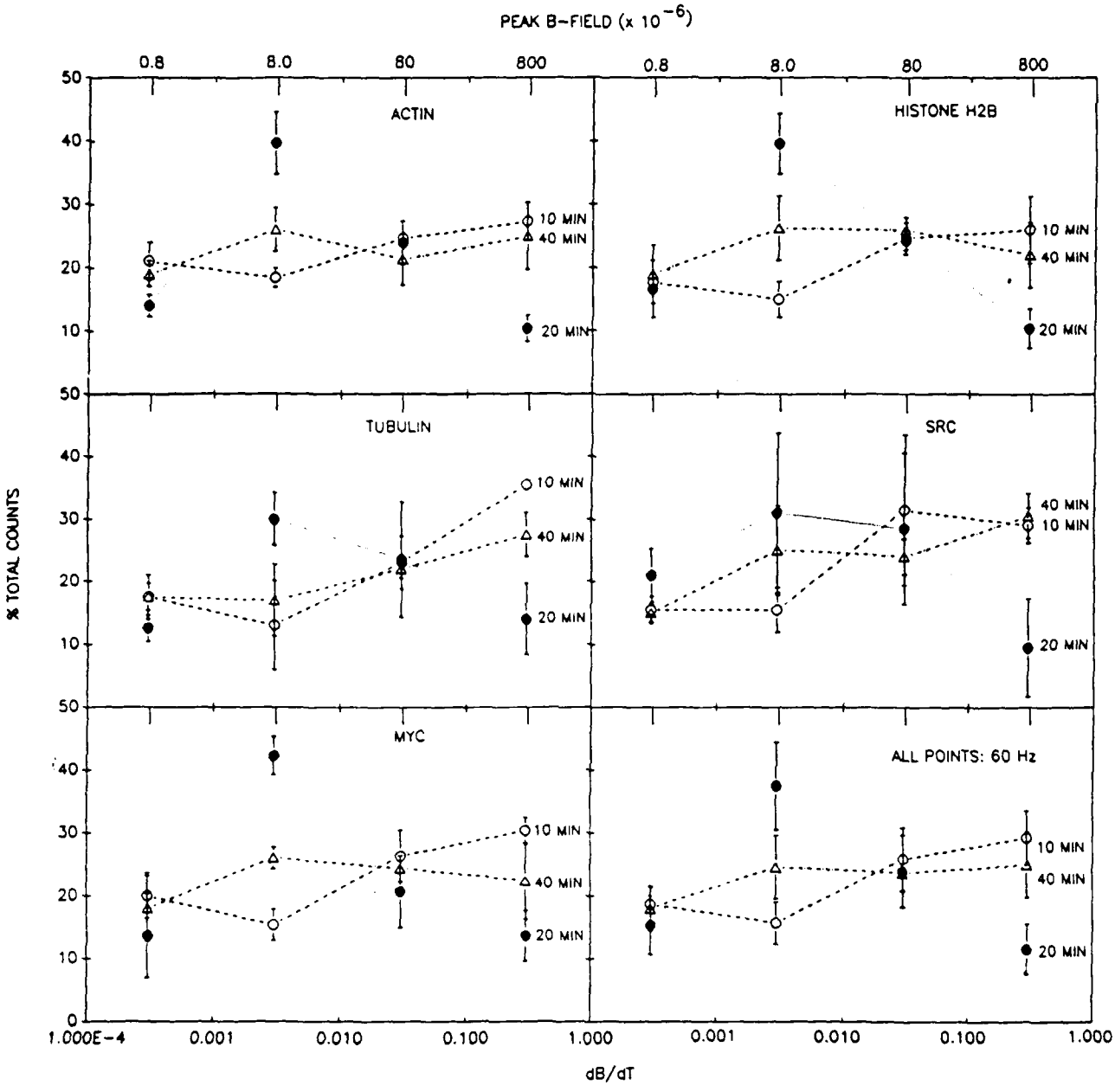

Figure 5. The effect of amplitude on transcripts in elf EMF- exposed HL60 cells. Each experiment represents a complete series; points were linear relative to RNA concentration. The data is compared as percent total counts for 2 ug RNA dilutions within an experimental series for inter-experimental comparisons, although the base level of transcripts was different. Three experiments are represented for myc, actin and histone hybridizations; two experiments for other probes; the final frame is all experiments. Bars are standard deviations. The x-axis is the rate of change of the magnetic field (dB/dT in T/sec) with increasing amplitude, where dB/dT at 0.5 uV is equal to 3 x 10^{-4} T/sec (from 26).

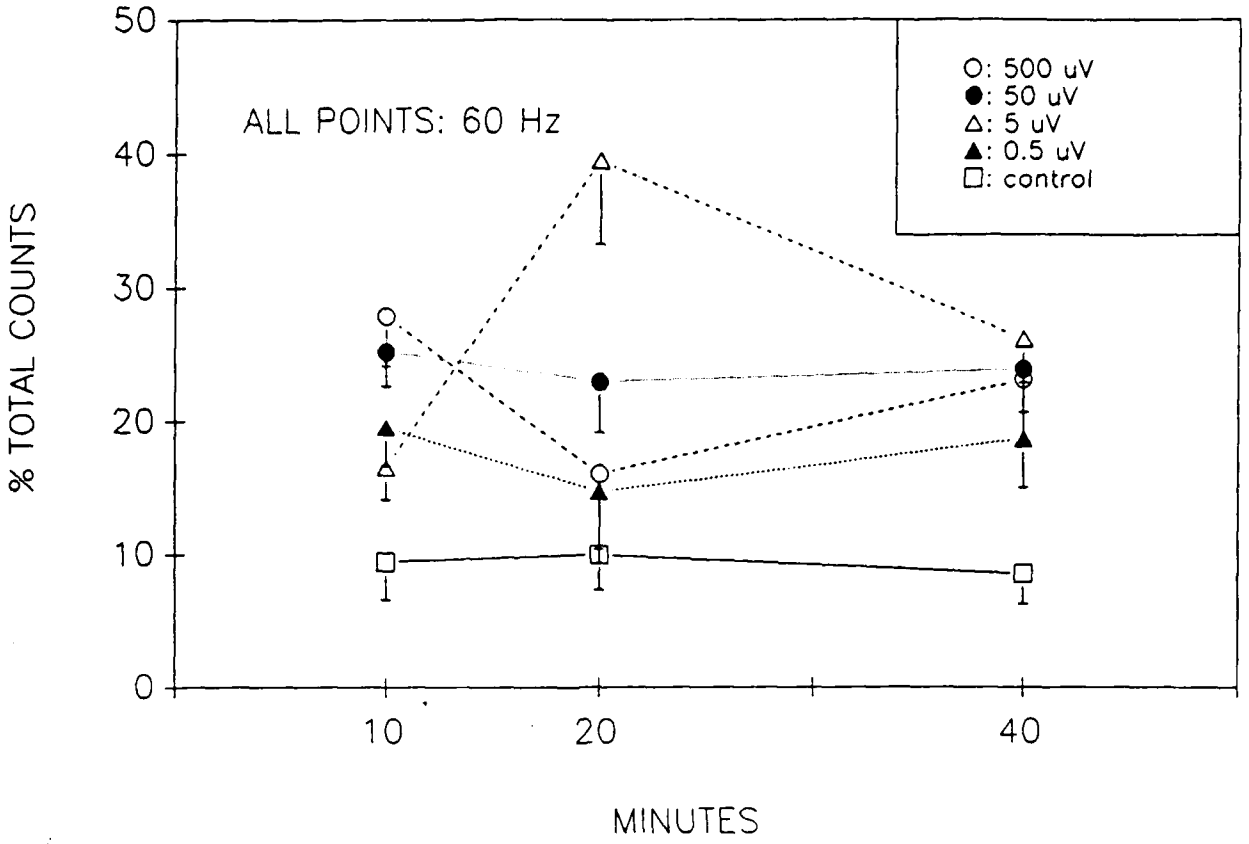

Figure 6. Analysis of quantity of transcripts following exposure of HL60 cells to the sinusoidal signals at 60 Hz for 10, 20 and 40 minutes. The data in Figure 5 were plotted to illustrate the time "window" (from 26)

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are exposed to 5 uV for 20 minutes. At 500 uV, however, transcript levels decrease to near control levels at 20 minutes exposure. The relative order for each point was invariant in all experiments, although there is some overlap in the standard deviations.

Conclusions. The present study shows that changing parameters in elf EMFs related to dose modulates the response of HL-60 cells. The response is probably restricted to genes expressed in these cells since there was no significant hybridization of the α-globin DNA. We do not know if elf EMFS affect all expressed genes, although this is probably not the case based on indirect evidence from analysis of proteins in 2-D gels (unpublished data). Our working hypothesis is that a subset of genes involved in growth and/or differentiation may be involved.

3.4 TRANSLATION-ELF EMFS AND PROTEIN SYNTHESIS. Previous results showed that exposure of Sciara (28) and Drosophila (in preparation) salivary gland cells to various types of elf EMFs, including 60 and 72 Hz sinusoidal signals, results in changes in the pattern of protein synthesis. 2-D gels were run using polypeptides from human HL60 cells exposed to 60 (two experiments) and 72 Hz (one experiment) sinusoidal waveforms at amplitudes of 0.5, 5,50 and 500 uV. Exposure to the signals was for 45 minutes, the minimal time for adequate radioactive labelling of the samples.

The first level of analysis has been completed, that of standardizing the samples (first and second level matched sets) for inter-experimental comparisons. The data is currently being analyzed using computer assistance. Preliminary results are given in Table 1 and Figure 7. Three features should be noted from the data. The first is that the correlation coefficients show that the experimental and control profiles are different. Second, quantitative differences are observed in selected molecular weight classes. Finally, we can demonstrate that the quantization of proteins in cells exposed to EMFs supports data which measured quantity of RNA transcripts. For example, the relative quantity of any transcript measured (at 40 minutes exposure) followed an order related to the signal: 50 uV $\geq$ 5uV $\geq$ 500 uV $\geq$ 0.5 uV $\geq$ control. One to two matched protein sets would show this order under random conditions. The number of matched sets in the present data having this order far exceeds that expected; 42/239 or about 17%; 26% results from sets where there was no effect relative to control values; other values were randomly distributed relative to exposure conditions (Table 1). This supports our hypothesis that a particular subset of transcripts is affected under these conditions of exposure.

4.0 CONCLUSIONS AND DISCUSSION

4.1. ELF EMFS AFFECT TRANSCRIPTION. The major question is how elf EMFs initiate events within a cell. To date, our research has approach the question of the conditions under which transcription within cells is affected. These studies demonstrate that cells respond to elf EMFs fields by increasing transcription in a manner of minutes. Transcription is affected by the nature of the signal (symmetrical vs. asymmetrical), as well as the time of exposure and other parameters such as changing the B- and/or E-fields. It is probable

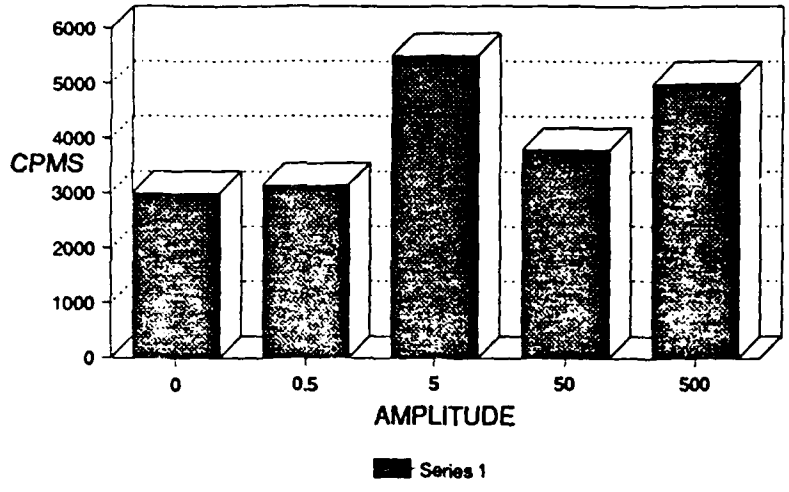

FIGURE 7. Incorporation of ^{35}S-methionine into the actin proteins as determined from 2-dimensional gel electrophoresis. Each amplitude (uV) was at 60 Hz (see figure 2)

TABLE 1

ANALYSIS OF 2-D GELS: PROTEINS IN CELLS EXPOSED TO 60 HZ FIELDS AT AMPLITUDES OF 0.5, 5, 50 AND 500 uV

Total matched sets for each parameter where the cpm > 100.	239
Matched sets where cpm at each parameter were approximately equal	26%
Matched sets where protein incorporation was as expected on the basis of transcription data (see Fig. 5)	17%
Random combinations-no apparent pattern	57%

that only those genes which are normally expressed in cells are affected by the presence of elf EMFs. Globin, a transcript not normally expressed in HL60 cells, is unaffected by elf EMF field exposure during the time periods we have tested. Using asymmetric signals provided no clue as to which specific physical parameters within the signals were responsible for the alteration in transcript quantity observed, or the mechanism that makes them effective in bone healing. Since the sinusoidal signals caused a greater increase in transcript quantity, and contains fewer physical parameters with which to be concerned, it becomes the signal of choice with which to pursue the elusive common interaction mechanism.

Some clue as to cellular interaction was obtained in the experiments where changing frequency and induced amplitude modulated the cellular response. Experiments testing transcript response to a frequency range revealed a window at 45 Hz when the amplitude was held constant at 500 uV. This result implies that at this frequency and amplitude, the magnetic flux or peak magnetic field is in some way implicated in the cellular response. Another window was revealed when the amplitude course was plotted. The most unexpected finding was that the response of cells to the exogenous elf EM fields resulted in an identical pattern of transcript augmentation. These data imply a general response on the part of the cell to an environmental stress. The critical experiments that should clarify mechanism will be those that define adaptation to time of signal exposure (minutes, hours etc) and those experiments in which cells that have adapted are exposed again to determine transcript response upon re-exposure.

4.2. CLUES TO MECHANISM-A GENERALIZED VIEW. In retrospect, an increase in transcription in cells exposed to elf EMFs is not a surprising finding in light of previous research in this field. There is general agreement that exogenous EMFs modify calcium transport, although the mechanism of modification is debatable (29-32). Frequency and intensity windows have been observed previously, suggesting synergism between non-ionizing radiation and the normal cell membrane potential. This idea is further supported by associated changes in calcium flux following exposure of cells to elf EMFs (24,33,34). Some investigators have concentrated primarily on electric fields on the assumption that there is amplification of the weak externally applied fields directly effecting primarily cell proteins or similarly charged structures. This has led to hypotheses which involve some function at

the cell membrane level via receptors, and incorporate the regulation of the efflux of calcium. Adey (35) has postulated that the critical initiating factors lay within the extracellular space (pericellular fluid) between cells, *i.e.*, the charge accumulation is in the vicinity of the cell membrane, resulting in cooperative processes involving proteins. Such signals could result in an amplification of a hierarchical cascade of energy steps, and set up transmembrane signaling as mediated by calcium or cyclic AMP. Luben (36,37) has shown that the receptor protein for parathyroid hormone is at least one site of field transduction. The fields did not alter binding to the specific receptor, but suggested an influence on transmission of the signal to the interior. The response was measured as reflected in the cyclic AMP (cAMP) accumulation and collagen synthesis.

Taken together, these data suggest that transduction mechanisms may be involved in the cellular response to EMFs. From a biologists point of view, a mechanism incorporating ideas of signal transduction makes sense. **It is also consistent with transcriptional activation**, and activation of specific inducible enzymes, such as ornithine carboxylase (35,38).

CLUES TO MECHANISMS-SPECIFIC PROBLEMS. The relationship of RNA synthetic activity in cells exposed to elf EMFs to DNA synthesis should be determined in our experiments. DNA synthetic activity in Chinese Hamster V79 cells and human fibroblasts is enhanced when the cells are exposed to pulsing electromagnetic fields for a wide range of frequencies (39,40). Whether short term exposures such as those used in the present experiments are initiating DNA synthetic activity has yet to be determined.

Experimental models developed using other inductive agents may also be ultimately useful in explaining the action of electromagnetic fields. For example, actin gene expression is known to be induced by a tumor-promoting phorbol ester (TPA) in the human cell line K562 (41) following one hour of treatment. The presence of the calcium ionophore A23187 and/or TPA induces a transient accumulation of c-*myc* in human lymphocytes (42), suggesting that the rise in c-*myc* transcripts is due to increase in the level of intracellular calcium.

A more prominent problem is the nature of the transcripts which respond to elf EMF signals. Critical to our analyses are how many total genes respond to EMFs. Is every expressed gene activated by exposure to elf EMFs or the more likely circumstance, only specific subsets? If

specific subsets of genes are implicated, it will simplify the task of determining if there are specific characteristics of gene sets that can be identified.

The frequency of initiation of mRNA synthesis depends on factors that interact with specific elements in the gene promoters. There is an assortment of regulatory elements both upstream and downstream of the RNA start site for the gene (reviewed in 43). These elements are binding sites for DNA sequence-specific protein transcription factors that activate or repress the activity of the gene. Each gene has a particular combination of positive and negative regulatory cis elements that are arranged as to number, type and spatial array. Overlapping or superimposed binding sites for multiple factors can result in different negative or positive factors competing for sites, and in some cases, synergistic effects that are dependent on the strict spacing between adjacent cis elements. The transcription factors that act of the promoters can be divided into at least two broad classes: at least 6 general transcription factors, and a much larger group of proteins, the promotor-selective (or promotor-specific) transcription factors. A large number of less common elements have been implicated in specialized types of signal-dependent transcription regulation, such as in response to heat shock, hormones or growth factors.

Information flow resulting from signal transduction (and other sources) can mediate the induction of transcription factors. The genes we have considered are those that are found in most cells, but the temporal appearance in the cell cycle for each is different. As DNA sequence information becomes available on the flanking regions for the genes we have monitored (β-actin, histone H2B, α-tubulin, v-src, α-globin and c-myc), and will monitor in the future, we should be able to delineated which factors are activated and under what conditions relative to elf EMF exposure.

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